actin filament sliding assay and a dual-beam, optical tweezers apparatus to perform single molecule mechanical studies. For these studies we used a two-headed, heavy meromyosin-like (HMM) construct of myosin-10 that contained a leucine-zipper at the C-terminal end to force dimerization. The actin filament gliding assay showed that myosin-10-HMM moves filaments at a velocity of ~125 nm s^{-1}. This is similar to the speed of intact, GFP-tagged, myosin-10 moving within filopodia of live mammalian cells (~140 nm s^{-1}) measured by TIRF microscopy. Optical trapping results showed that the average power-stroke size was ~10 nm, with the rate of ATP binding of ~1.3 μM^{-1} s^{-1}. In most of the raw data traces, we observed displacements of unitary size, however at low ATP concentrations (2 μM) we also observed a number of interactions that exhibited multiple, staircase-like movements consisting of up to 3 steps per binding interaction. This behaviour is characteristic of a *processive* molecular motor. We will discuss these measurements in the context of mechano-chemical coupling and the functional significance in the living cell.

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Bayesian Estimation for Hidden Information of a Single Molecular Motor

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In single-molecule experiments of molecular motors, it is a widely adopted strategy to visualize the motion of a molecule by attaching a large probe particle. In such a situation, only the motion of the probe can be monitored while the motion of the molecule is hidden. Therefore, in order to study a stepwise motion of the motor in detail, one has to estimate the system parameters and the hidden trajectory of the motor molecule at the same time on the basis of the observed trajectory of the probe. In this presentation, we will present a novel approach for this problem, which is based on the statistical mechanics and empirical Bayesian estimation. The method consists of several steps. First, we temporarily fix the parameters of the system. Then, we can utilize the Bayes theorem to estimate the trajectory of the motor from the trajectory of the probe. On the basis of the estimated trajectory, the marginal likelihood can be calculated using the WKB method. Finally, by maximizing the marginal likelihood, we can estimate the system parameters along with the trajectory of the motor. Although the above mentioned tasks appear straightforward, it is necessary to adopt appropriate approximation schemes in order to reduce the computational cost as well as artifacts resulting from the finite time resolution of the measurements. It is found that our method gives a reasonable solution to this problem. We will discuss the effectiveness of the method presented here by using a simple model that consists of two Brownian particles (a molecule and a probe) connected by a spring.

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ADP Affinity of Myosin VI is Regulated by Off-Axis Load

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Individual dimeric molecules of unconventional myosin VI move processively along an actin filament, taking multiple steps in a hand-over-hand fashion. Recently we showed experimentally that the efficient processive movement is achieved via asymmetric rates of ADP binding under the forward and the backward loads, that is, to the trailing and the leading heads, respectively, such that ADP dissociates more readily from the trailing head, though the difference in the ADP affinity between the heads is less pronounced than in case of myosin V-6IQ construct. Several lines of evidence, obtained by other groups, suggest that during the processive stepping both the azimuthal angle of myosin's VI lever arm and the path of the motor along an actin filament are very variable, which implies the existence of an off-axis load during the double-headed binding of myosin VI to actin. To reveal the effect of an off-axis load on the efficiency of the processive stepping, we directly measured the ADP affinity of individual single-headed myosin VI molecules bound to actin, under loads applied at certain angles to the long axis of actin filament, towards both barbed and pointed ends. The obtained results indicate that the off-axis load significantly affects ADP affinity of myosin VI motor domain and is an important factor regulating the efficient processive movement of the dimeric molecules of myosin VI.

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The Tail Binds To The Head-Neck Domain To Form A Folded-Back Conformation That Inhibits The Actin-Activated ATPase Activity Of Drosophila Myosin VIIA

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Myosin VIIA is an unconventional myosin, responsible for human Usher Syndrome type 1B, which causes hearing and visual loss. We have analyzed the molecular mechanism of regulation of myosin VIIA, which is not yet understood. While it was originally thought that myosin VIIA is a dimeric myosin, our electron microscopic (EM) observations revealed that full-length Drosophila myosin VIIA (DM7A) is monomeric. Interestingly, the tail domain markedly inhibits the actin-activated ATPase activity of tail-less DM7A in the absence of Ca2+ but not below pCa 6. By examining various deletion constructs, we found that deletion of the distal IQ domain, the C-terminal domain of the tail, or the N-terminal domain of the tail abolishes the tail-induced inhibition of ATPase activity.

EM study of full-length DM7A suggests that the tail domain folds back on to the head, such that the tail and the head/neck domain contact each other. Single particle analysis of full-length DM7A and of tail-less molecules to which exogenous tail has been added reveals that the tail contacts the head/neck domain at two sites, the motor core domain and the neck domain. These observations, together with the functional results, suggest that DM7A is inactivated by forming an inhibited conformation, in which the tail interacts with the motor and lever-arm domains. This is the first report to identify the regulatory mechanism of a single-headed unconventional myosin.

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The Medial-tail Domain of Myosin-VI as a Dimerization Region

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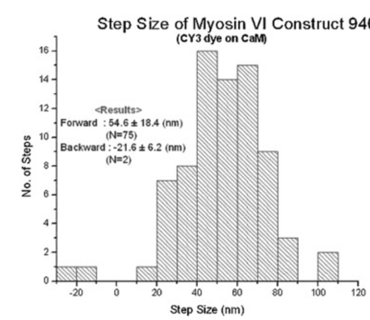
Myosin-VI has been one of the least-understood unconventional molecular motors due to its peculiar characteristics. For instance, each myosin-VI monomer has a short lever arm (~10nm) which contains only two Calmodulins while taking large steps (~36nm center-of-mass movements). Recently, the Spudich group proposed, based on bacterial expressed fragments, that the medial-tail domain of myosin-VI is a single alpha helix (~10nm), which can account for its large step sizes if dimerization occurs after the medial-tail domain. They also suggested that dimerization is formed via the cargo-binding domain. However, these results are contrary to our previous paper that showed that a myosin-VI construct without the cargo-binding domain can dimerize and walk processively. To solve this paradox, we prepared a myosin-VI construct truncated in the middle of the medial-tail domain (940-construct). A FIONA assay showed that this construct does dimerize either via antibody induction or actin-saturation method, and it walks processively with the same step size as the full-length myosin-VI construct. These results suggest that the medial-tail domain of myosin-VI is involved in dimerization and imply that the proximal-tail domain must be the major contributor to the unexpectedly large step size.



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Processivity of Myosin V and X on two-dimensional (2D) paracrystalline actin array

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Myosin movement in vivo takes place on a wide variety of F-actin structures, including single filaments and 2D/3D bundled networks. Using in vitro single molecule motility techniqes, we have investigated the processivity and stepping characteristics of myosin V HMM and myosin X HMM with a leucine zipper on single actin filaments and 2D actin bundles. To answer how myosin V and myosin X step on actin bundles, we observed single molecule motility of fluorescently labeled myosin V and X using total internal reflection fluorescent microscopy, and analyzed the step-size, run length, speed, and direction of the movements on actin-bundles. Actin was polymerized and cross-linked on a charged lipid monolayer in Teflon wells to create regular 2D actin arrays. Two cross-linking proteins were used: alpha-actinin, which produces non-polarized bundles with 40 nm filament spacing, and fascin